

## KINESIN AND SPERMATID DIFFERENTIATION IN MARSILEA VESTITA

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The microspore of the water fern *Marsilea vestita*, contains a single cell that undergoes a series of nine successive mitotic divisions to produce 32 spermatids, six jacket cells and one sterile prothallial cell. Immediately after the dry microspore is placed into water, significant movement within the cytoplasm precedes the first division; certain proteins and mRNAs aggregate into zones that later become the spermatogenous initials of the gametophyte. Our working hypothesis is that the asymmetric redistribution of proteins and mRNAs is driven by microtubule-based motility systems. Kinesin-driven movements along cytoplasmic microtubules appear to be involved in this process. Our goal is to study how kinesin affects development in particular stages of spermiogenesis. We developed RNAi strategies to target the degradation of specific mRNAs to arrest development in the gametophyte. These published studies show that the pattern of translation in the gametophyte is precisely ordered, which indicates that certain proteins are required at specific stages of development. In the current study, dsRNA probes were made from a cDNA encoding kinesin-like protein isolated from our gametophyte library. dsRNAs made from this cDNA were added to populations of developing gametophytes to assess the time and stage of development at which this transcript becomes limiting. Development is arrested prior to the completion of all nine division cycles. Treated cells were then labeled with anti-centrin and anti- $\beta$ -tubulin antibodies to determine the stage of arrest and the effect of a kinesin null phenocopy on the known distributions of centrin and  $\beta$ -tubulin proteins. The patterns of developmental disruption induced by the absence of this kinesin-like protein are readily discernable in cells fixed at various times during development and precede the onset of de novo basal body formation, cell and nuclear shaping, and ciliogenesis. (Supported by NSF grant MCB 0234423 to SMW).